abcam

## ab16665

# Anti-p53 antibody [SP5]

Anti-p53 antibody [SP5] (ab16665) is a rabbit monoclonal antibody detecting p53 in **Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Trusted since 2005

Lab Essentials | Recombinant | RabMAb | KO Validated | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | SP5 |
| **Purification technique** | Affinity purification Protein A |
| **Epitope** | Not determined |
| **Concentration** | 0.004 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## IHC-P

**Tested**

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/100 |
| **Notes** | Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/200 |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/25 - 1/100 |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/500 |
| **Notes** | - |

## IP

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 2 µg |
| **Notes** | - |

# Target data

See full target information TP53

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|---|---|
| **Function** | Multifunctional transcription factor that induces cell cycle arrest, DNA repair or apoptosis upon binding to its target DNA sequence (PubMed:11025664, PubMed:12524540, PubMed:12810724, PubMed:15186775, PubMed:15340061, PubMed:17317671, PubMed:17349958, PubMed:19556538, PubMed:20673990, PubMed:20959462, PubMed:22726440, PubMed:24051492, PubMed:24652652, PubMed:35618207, PubMed:36634798, PubMed:38653238, PubMed:9840937). Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type (PubMed:11025664, PubMed:12524540, PubMed:12810724, PubMed:15186775, PubMed:15340061, PubMed:17189187, PubMed:17317671, PubMed:17349958, PubMed:19556538, PubMed:20673990, PubMed:20959462, PubMed:22726440, PubMed:24051492, PubMed:24652652, PubMed:38653238, PubMed:9840937). Negatively regulates cell division by controlling expression of a set of genes required for this process (PubMed:11025664, PubMed:12524540, PubMed:12810724, PubMed:15186775, PubMed:15340061, PubMed:17317671, PubMed:17349958, PubMed:19556538, PubMed:20673990, PubMed:20959462, PubMed:22726440, PubMed:24051492, PubMed:24652652, PubMed:9840937). One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression (PubMed:12524540, PubMed:17189187). Its pro-apoptotic activity is activated via its interaction with PPP1R13B/ASPP1 or TP53BP2/ASPP2 (PubMed:12524540). However, this activity is inhibited when the interaction with PPP1R13B/ASPP1 or TP53BP2/ASPP2 is displaced by PPP1R13L/iASPP (PubMed:12524540). In cooperation with mitochondrial PPIF is involved in activating oxidative stress-induced necrosis; the function is largely independent of transcription. Induces the transcription of long intergenic non-coding RNA p21 (lincRNA-p21) and lincRNA-Mkln1. LincRNA-p21 participates in TP53-dependent transcriptional repression leading to apoptosis and seems to have an effect on cell-cycle regulation. Implicated in Notch signaling cross-over. Prevents CDK7 kinase activity when associated to CAK complex in response to DNA damage, thus stopping cell cycle progression. Isoform 2 enhances the transactivation activity of isoform 1 from some but not all TP53-inducible promoters. Isoform 4 suppresses transactivation activity and impairs growth suppression mediated by isoform 1. Isoform 7 inhibits isoform 1-mediated apoptosis. Regulates the circadian clock by repressing CLOCK-BMAL1-mediated transcriptional activation of PER2 (PubMed:24051492). |

# Storage

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| **Shipped at conditions** | Conditional Ambient |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |

| | |
|---|---|
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

**What is this antibody validated in?**
Anti-p53 antibody [SP5] (ab16665) is a rabbit recombinant monoclonal antibody and is validated for use in Flow Cytometry (Intra), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

**Collaboration**
Anti-p53 [SP5] (ab16665) is a clone from the portfolio of Spring Bioscience (Roche) SP clones which have been optimised for immunohistochemistry (IHC).

**Specificity confirmed**
The specificity of Anti-p53 antibody [SP5] (ab16665) has been confirmed by Immunocytochemistry testing in TP53 Knockout HAP1 cells.

**Other related products**
We have a range of other formats of antibody clone [SP5] also available for your convenience: ab16665, ab27598, ab238806, Carrier free - ab242379, Alexa Fluor® 488 - ab270192, ab279603

**What are the advantages of a recombinant monoclonal antibody?**
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

**Trial sizes available!**
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | The protein p53 also known as TP53 or tumor protein p53 has a molecular weight of approximately 53 kDa. It acts as a transcription factor and plays a major role in cell cycle regulation apoptosis and maintaining genomic stability. This protein mainly expresses in the nucleus of cells and acts as a critical regulator of cellular responses to stress signals including DNA damage. Scientists commonly use p53 |

| | |
|---|---|
| | antibodies in various assays like western blot and p53 immunofluorescence to detect and study its expression and functional status in cells. |
| **Biological function summary** | P53 functions to control cell division and apoptosis serving as a guardian of the genome by preventing mutation accumulation. It does not form part of a larger complex under normal conditions but interacts with various other molecules to execute its functions. p53 can activate or suppress the transcription of numerous genes involved in cell cycle arrest DNA repair and programmed cell death allowing it to halt the progression of damaged cells and trigger repair mechanisms or eliminate those that cannot be repaired. |
| **Pathways** | P53 acts within several key biological pathways such as the p53 signaling pathway and the intrinsic apoptotic pathway. Its activity involves interaction with proteins like MDM2 which regulates p53 through ubiquitin-mediated degradation and ATM kinase which phosphorylates p53 in response to DNA damage. These interactions ensure appropriate cellular responses during stress and are vital for maintaining homeostasis. |
| **Associated diseases and disorders** | P53 mutation or inactivation is often associated with the development of cancer given its role in controlling cell division and preventing tumor formation. Specifically its dysfunction has been linked to cancers such as breast cancer and lung cancer. Additionally p53 can interact with other mutant proteins like Ras compounding mutations that contribute to tumor progression and aggressive cancer phenotypes. Understanding these interactions and the status of p53 can be important in developing targeted cancer therapies. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

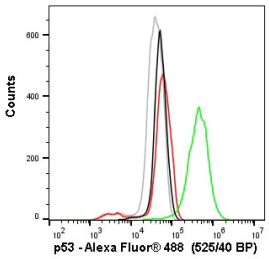
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

## 9 product images




### Flow Cytometry (Intracellular) - Anti-p53 antibody [SP5] (ab16665)

P53 Flow Cytometry (Intracellular) staining using rabbit Anti-p53 antibody

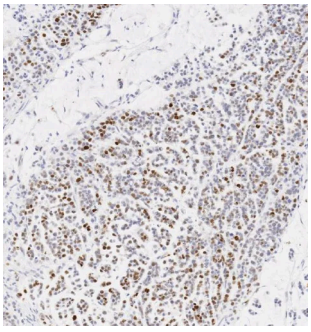
Flow cytometry overlay histogram showing wild-type HAP1 (green line) and TP53 knockout HAP1 cells stained with ab16665 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1%PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab16665) ($1x10^6$ in 100 µl at 0.008 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluor® 488, pre-adsorbed) (ab150081) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody was Rabbit IgG (monoclonal) (ab172730) used at the same concentration and conditions as the primary antibody (wild-type HAP1 - black line TP53 knockout HAP1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

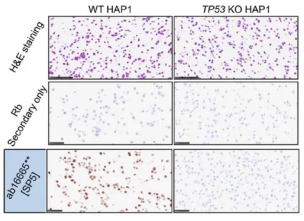
This antibody gave a positive signal in TP53 HAP1 knockout cells fixed with 80% methanol (5 min) / permeabilized with 0.1%PBS-Triton X-100 for 15 min used under the same conditions.



### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [SP5] (ab16665)

P53 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-p53 antibody

Formalin-fixed, paraffin-embedded human colon carcinoma tissue stained for p53 using ab16665 at 1/100 dilution in immunohistochemical analysis.



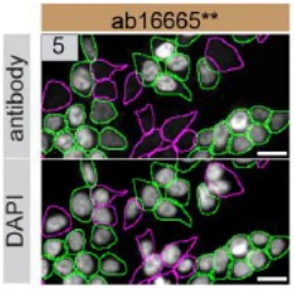

### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [SP5] (ab16665)

P53 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of wild-type HAP1 cells using rabbit Anti-p53 antibody

ab16665 was shown to react with TP53 in wild-type HAP1 cells in immunohistochemistry with loss of signal observed in a TP53 knockout cell line. HAP1 WT and TP53 KO cells were fixed and paraffin embedded. Sections were mounted onto slides and stained with ab16665 and the corresponding multimer HRP secondary. Chromogen image acquisition was performed, and representative images of each section are shown. Scale bar = 50 µm.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and

YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

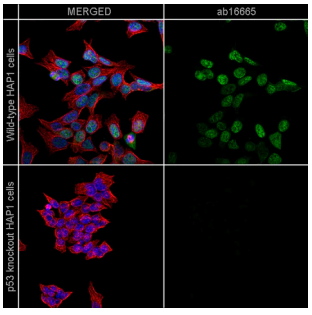



## Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [SP5] (ab16665)

P53 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p53 antibody

ab16665 was shown to react with TP53 in wild-type HAP1 cells in immunocytochemistry with loss of signal observed in a TP53 knockout cell line. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1x PBS, 0.01% Triton X-100, 5% BSA, 5% NGS. The cells were then incubated with ab16665 at 1/100 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880).

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

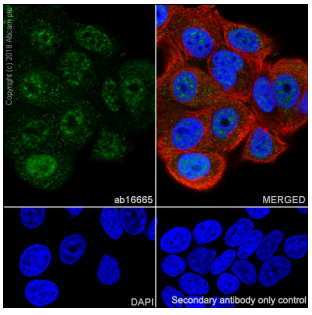



## Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [SP5] (ab16665)

P53 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p53 antibody

ab16665 staining p53 in wild-type Hap1 cells (top panel) and p53 knockout Hap1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab16665 at 1/25 dilution and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a high-content analysis system (Perkin Elmer, Operetta CLS™).

This image was generated from the hybridoma version of the product

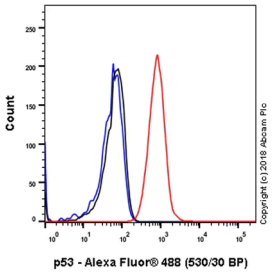



## Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [SP5] (ab16665)

P53 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p53 antibody

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (human breast adenocarcinoma epithelial cell) cells labeling p53 with purified ab16665 at 1/25 (6.5 µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at

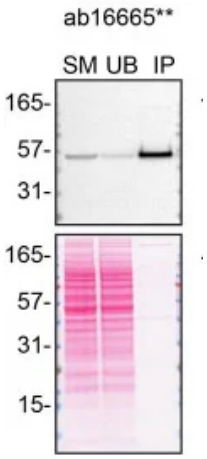
1/1000 (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This image was generated from the hybridoma version of the product




## Flow Cytometry (Intracellular) - Anti-p53 antibody [SP5] (ab16665)

P53 Flow Cytometry (Intracellular) staining using rabbit Anti-p53 antibody

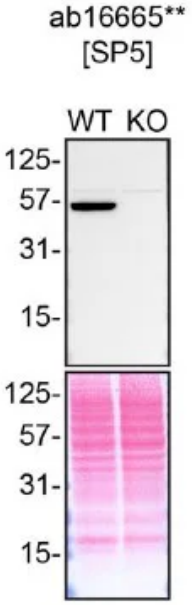
This image was generated from the hybridoma version of the product
Flow cytometry analysis of MCF7 (human breast adenocarcinoma epithelial cell) labeling p53 with purified ab16665 at 1/200 dilution (0.81 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal IgG (ab172730) (black). Unlableled control - Unlabelled cells (blue).




## Immunoprecipitation - Anti-p53 antibody [SP5] (ab16665)

Immunoprecipitation of TP53 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 2 µg of ab16665 pre-coupled to Protein A beads. Samples were then washed and processed for western blot.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.




## Western blot - Anti-p53 antibody [SP5] (ab16665)

P53 Western blot staining using rabbit Anti-p53 antibody

ab16665 was shown to react with TP53 in wild-type HAP1 cells in Western blot with loss of signal observed in a TP53 knockout cell line. Wild-type HAP1 and TP53 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab16665 overnight at 4 °C at a 1/500 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Western blot - Anti-p53 antibody [SP5] (ab16665) at 1/500 dilution

Lane 1:
Wild-type HAP1 lysate at 20 µg

Lane 2:
TP53 knock-out HAP1 lysate at 20 µg

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.